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# Riboflavin and rose bengal sensitised photooxidation of sulfathiazole and succinylsulfathiazole Kinetic study and microbiological implications

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**Abstract**

In the presence of the synthetic xanthenic dye rose bengal or the pigment riboflavin, the bacteriostatics sulfathiazole (**STZ**) and succinylsulfathiazole (**SCSTZ**) suffer visible-light promoted degradation in dilute aqueous and aqueous-ethanolic solutions. The events occur to a different extent, depending on the chemical structures of the thiazoles and pH of the medium. The photo-oxidation process is accompanied by a partial loss of the antibacterial activity of the thiazoles. The extent of photodegradation occurs quickly under alkaline conditions and is less aggressive in the physiological pH region, although not negligible in kinetic terms. Photo-oxidation quantum efficiencies, evaluated through singlet molecular oxygen [$O_2(^1\Delta_g)$] phosphorescence detection, spectrophotometric and polarographic methods, range from 0.002 to 0.31 as the upper limit in alkaline media. With rose bengal as a sensitiser in aqueous medium, photo-oxidation essentially proceeds via singlet molecular oxygen. The results indicate the occurrence of several photo-processes that involve a series of competitive reactions, including singlet molecular oxygen photo-oxidation, when the vitamin riboflavin was employed as a sensitiser, in a 1:1 water: ethanol medium. Their relative importance depends on the concentration of the thiazoles and riboflavin. The bacteriostatics efficiently quench both the excited singlet and triplet states of the pigment. The final result of the interactions is the phototransformation of the thiazoles and the partial photostabilization of riboflavin. The $O_2(^1\Delta_g)$-mediated photo-oxidation of **STZ** produces a decrease in its bacteriostatic properties. 

*Keywords:* Photodegradation; Photo-oxidation; Riboflavin; Rose bengal; Succinylsulfathiazole; Sulfathiazole

## 1. Introduction

Dye-sensitised photo-oxidation is being increasingly investigated in relation to substrates of relevance in photobiology and photomedicin [1,2]. Antibiotics and bacteriostatics, which are generally transparent to direct natural light photo-irradiation, belong to this class of biologically active and commercially valuable substrates. In these domains, the most frequently studied photo-reactions are those mediated by singlet molecular oxygen [$O_2(^1\Delta_g)$], through the so-called Type II photoprocess [3]. In these cases the only requirement is the coincidence of dissolved oxygen,

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daylight and a daylight-absorbing impurity in the medium containing the antibiotic, a scheme which is largely fulfilled under natural ambient conditions. In past decades, several soluble and insoluble synthetic dyes and natural pigments have been analysed [4–7] in connection with their prooxidant properties through the photogeneration of the reactive species $O_2(^1\Delta_g)$. However, we are not aware of studies that concern dye-sensitised photo-oxidation of the widely employed bacteriostatic [8] sulfathiazole (**STZ**) and succinylsulfathiazole (**SCSTZ**). Their respective structural formulae are given in Chart 1.

The sensitisers chosen for this study were the natural product riboflavin (Rf) and a synthetic one as the xanthene derivative rose bengal (RB). In the presence of oxygen, flavins in general and Rf in particular, are known to photosensitise the decomposition of a variety of biologically relevant substances through a broad spectrum of mechanistic possibilities. Rose bengal is an excellent sensitiser for $O_2(^1\Delta_g)$ reaction [4] and was chosen in order to selectively separate for the study the contribution from this excited oxygen species to the overall reaction mechanism.

As complementary parallel work, the antimicrobial activity of the above mentioned compounds as a result of dye-sensitised photoirradation was determined, in order to establish the extent of the photodegradation effect on the microbiological behaviour.

## 2. Experimental

### 2.1. Chemicals

Rose bengal (RB), furfuryl alcohol (FFA), sulfathiazole (**STZ**), succinylsulfathiazole (**SCSTZ**), thiazole (**TZ**) and riboflavin (Rf) were purchased from Sigma Chem. Co. Ethanol 97% was obtained from Sintorgan, Argentina; water was triply distilled. The pH values 6 and 12 were secured using phosphate buffer [9]. All thiazoles employed in this work will be generically named **Q** hereafter.

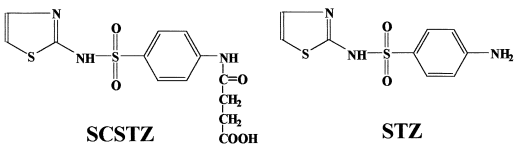



Chart 1.

### 2.2. Methods

Static and time-resolved fluorescence. Stationary Rf fluorescence quenching experiments were carried out at room temperature in air equilibrated solutions ($25 \pm 1^{\circ}C$) using a Spex Fluoromax spectrofluorimeter. Fluorescence lifetimes were measured with a time-correlated single photon counting technique employing an Edinburgh FL-9000CD instrument. In both cases, excitation and emission wavelengths were 445 and 515 nm, respectively. Ground state absorption spectra were registered in a Hewlett–Packard 8452A diode array spectrophotometer.

#### 2.2.1. Laser flash photolysis experiments

Nitrogen-saturated 0.01 mM Rf or RB aqueous solutions were photolysed using flash photolysis apparatus with the frequency-doubled output of a Nd:YAG laser (Spectron) at 532 nm as excitation wavelength, employing a 150 W xenon lamp as analysing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital osciloscope (Hewlett–Packard 54504A), was transferred to a PC via a HPIB parallel interface, where it was analysed and stored.

Static photolysis was carried out using a 150 W quartz-halogen lamp fitted with a 400 nm cut-off filter. The photolyser and the specific oxygen electrode have been described elsewhere [10].

#### 2.2.2. Stationary photolysis

The reactive rate constants $k_r$ (see kinetic scheme) for the reaction of the thiazoles with $O_2(^1\Delta_g)$ were determined using the method described by Scully and Hoigné [11] (Eq. (1)), for which a knowledge of the reactive rate constant for the photooxidation of a reference compound R is required:

$$\text{slope}_{Q}/\text{slope}_{\text{Reference}} = k_{rQ}/k_{r\text{Reference}} \quad (1)$$

where $\text{slope}_{Q}$ and $\text{slope}_{\text{Reference}}$ are the respective slopes of their pseudo first order plots of **Q** and reference consumption respectively upon sensitised irradiation. The reference compound and **Q** were used at identical concentration; oxygen uptake in water was monitored, instead of substrate consumption. Assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher is the only means of oxygen consumption, the ratio of the first order slopes of oxygen uptake by the reference compound and the substrate, each at the same concentration (slope reference/slope substrate) yields $k_r/k_{rR}$. The reference was FFA, with a reported [3] $k_r = 1.2 \times 10^8\ M^{-1}\ s^{-1}$ .

Ground state absorption measurements were carried out with a Hewlett–Packard 8452A diode array spectrophotometer.

### 2.2.3. Time resolved $O_2(^1\Delta_g)$ phosphorescence detection (TRPD)

The laser-kinetic spectrophotometer employed for the determination of $k_t$ has been previously described [12]. Briefly, it consisted in a Nd:Yag laser (Spectron) as the excitation source. The frequency-doubled output at 532 nm was employed to excite RB. The emitted radiation [$O_2(^1\Delta_g)$ phosphorescence, 1270 nm] was detected at right angles using an amplified Judson J16/8Sp germanium detector, after having passed through appropriate filters. The output of the detector was coupled to a digital oscilloscope and to a personal computer to carry out the signal processing. Usually, 16 shots were needed for averaging so as to achieve a good signal/noise ratio, from which the decay time was calculated. Air-equilibrated solutions were employed in all cases.

The absorbance of the sensitiser was 0.3 at the lasing wavelength. The $O_2(^1\Delta_g)$ lifetimes in MeCN–$D_2O$ and $D_2O$ were 55 and 60 μs, respectively. The solvent $D_2O$, instead of $H_2O$, was employed in the dynamic determinations in order to enlarge the lifetime of $O_2(^1\Delta_g)$, as already discussed elsewhere [13].

The $O_2(^1\Delta_g)$ lifetimes were evaluated in the absence $(\tau^0)$ and in the presence $(\tau)$ of the quencher and the data was plotted as a function of substrate concentration, according to a simple Stern–Volmer treatment (Eq. (2)).

$$1/\tau = 1/\tau^0 + k_t[Q] \quad (2)$$

### 2.2.4. Bacteriostatic activity

The bacteriostatic activity of 0.5 mM **STZ** solutions was evaluated at different irradiation times, employing the Kirby–Bauer methodology [14], according to Farmacopeia Nacional Argentina [15] and the norms of the document M2-A4 of the National Commitee for Clinical Laboratory Standard [16], using the standard stamp *Bacillus subtilis* (strain ATCC 6633). It was assayed in logarithmic phase on a Petris' plate employing the Mueller–Hilton culture medium. The virgin disks for antibacterial test were generously provided by the Britannia Company (Argentina).

## 3. Results

### 3.1. Kinetic scheme

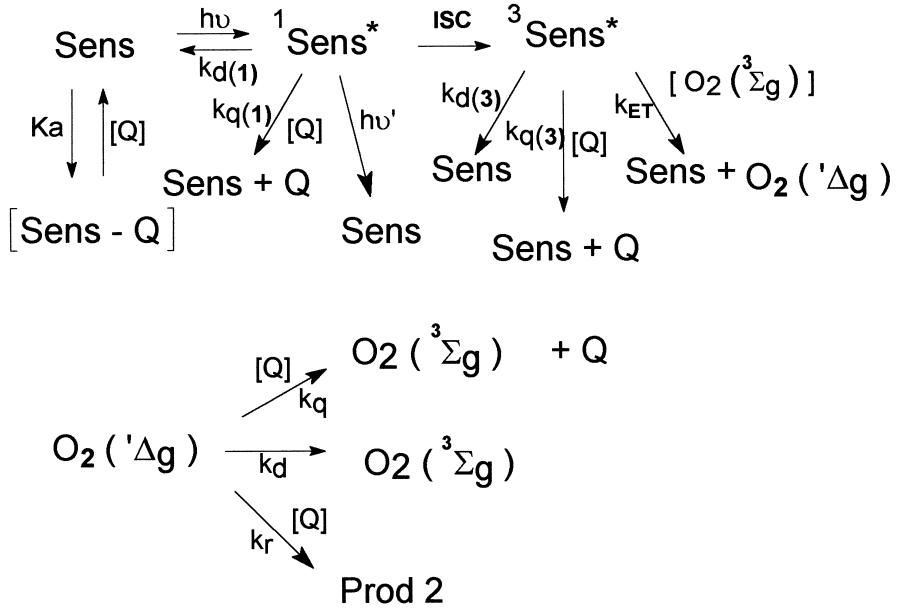
The kinetic steps employed for the evaluation and discussion of the experimental results are shown in Scheme 1, where $k_{q(1)}$ and $k_{q(3)}$ represent the rate constants for the quenching of the excited singlet and triplet states of the sensitisers by the thiazoles (**Q**), respectively. The rate constants $k_q$, and $k_r$ account for physical and reactive or chemical quenching of $O_2(^1\Delta_g)$ by the thiazoles, respectively, and $k_d$ (process 7) is the rate constant of the natural deactivation of $O_2(^1\Delta_g)$.

### 3.2. Aerobic RB-sensitised photo-irradiation of the thiazoles

The visible-light irradiation of air-equilibrated individual aqueous solutions of **STZ** and **SCSTZ** in the presence of RB (absorbance at 530 nm $\approx 0.5$) strongly modifies the absorption spectra of the thiazoles Figs. 1A and 1B. The magnitude of the spectral modifications for a given irradiation time were different at the different pH values used. The reactivity under alkaline conditions was much higher than that observed in slightly

acidic solutions. No reactivity at all could be observed for solutions containing the sensitiser and **TZ**.

The changes discussed above were markedly inhibited by the presence of 10 mM sodium azide, a well-known [3] physical quencher of $O_2(^1\Delta_g)$. These results indicate a Type II photo-oxidation, which was unambiguously corroborated by the $O_2(^1\Delta_g)$ -quenching experiments with TRPD.




Scheme 1.

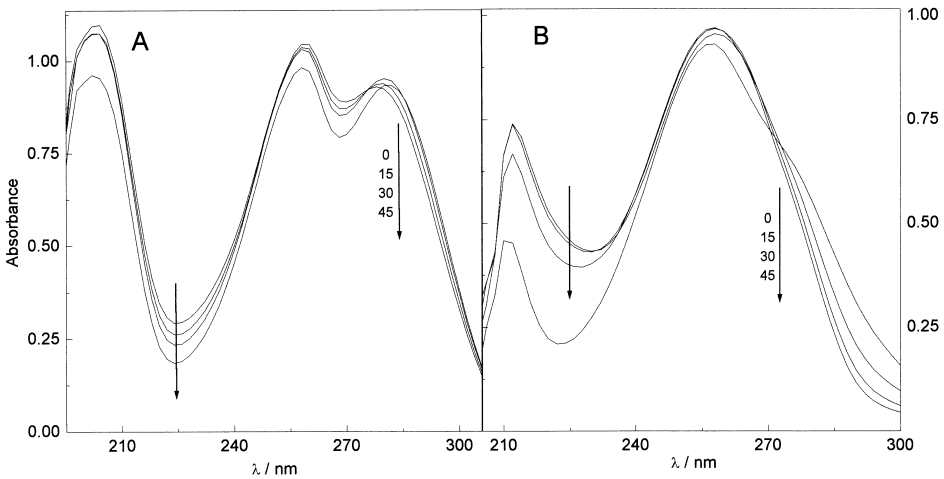



Fig. 1. Spectral changes for the RB-sensitised photo-oxidation of succinylsulfathiazole in air-equilibrated aqueous solution. Panel A at pH 6 and Panel B at pH 12. Absorbance of the sensitiser at 548 nm 0.5, respectively. Numbers on the spectra represent irradiation time in seconds.

Table 1
Rate constants for reactive ($k_r$, $M^{-1}$ $s^{-1}$) and overall ($k_t$, $M^{-1}$ $s^{-1}$) quenching of singlet molecular oxygen by sulfathiazole and succinylsulfathiazole, quantum efficiencies for singlet molecular oxygen-mediated photo-oxidation ($\varphi_r$) of the thiazoles and for the quenching of excited singlet state ($k_{q(1)}$, $M^{-1}$ $s^{-1}$) and excited triplet state ($k_{q(3)}$, $M^{-1}$ $s^{-1}$) of riboflavin by the thiazoles

| Compound | Solvent | $k_r \times 10^8$ | $k_t \times 10^8$ | $\varphi_r$ | $k_{q(1)} \times 10^8$ | $k_{q(3)} \times 10^8$ |
|---|---|---|---|---|---|---|
| Sulfathiazole | $H_2O$, pH 6 | 0.42 | | 0.16 | | |
| | $D_2O$, pD 6 | | 0.58 | | | |
| | $H_2O$, pH 12 | 0.52 | | 0.14 | | |
| | $D_2O$, pD 12 | | 1.07 | | | |
| | EtOH–$H_2O$ 1:1 | | | | 22[a]; 25[b] | 17 |
| Succinylsulfathiazole | $H_2O$, pH 6 | 0.006 | | 0.002 | | |
| | $D_2O$, pD 6 | | 0.2 | | | |
| | $H_2O$, pH 12 | 1.15 | | 0.31 | | |
| | $D_2O$, pD 12 | | 1.15 | | | |
| | EtOH–$H_2O$ 1:1 | | | | 17[a] and [b] | 6.9 |

[a] Determined by static fluorescence.
[b] Determined by time-resolved fluorescence.

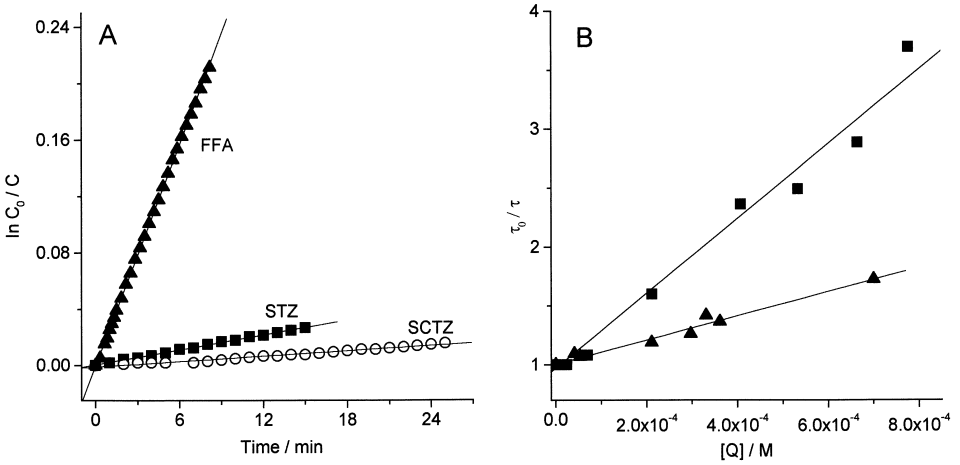



Fig. 2. Panel A: pseudo first order plots for oxygen uptake by 0.4 mM sulfathiazole, succinylsulfathiazole and furfuryl alcohol (the reference) in water, pH 6, upon rose bengal-sensitised photo-oxidation. Panel B: Stern–Volmer plots for the quenching of singlet molecular oxygen phosforescence, by sulfathiazole (■) and succinylsulfathiazole (▲) in water, pH 6.

### 3.3. Quenching of $O_2(^1\Delta_g)$ by *STZ* and *SCZTZ*

The $k_r$ values for **Q** (Table 1), were obtained through measurement of oxygen uptake, as shown in Fig. 2 A for typical cases. Oxygen consumption upon irradiation of aerated RB–**Q** was totally inhibited by the presence of 0.01 M $NaN_3$.

The treatment of the data obtained by means of the TRPD method, Eq. (2) for **Q** is shown in Fig. 2b. Table 1 contains the respective $k_t$ values obtained in $D_2O$, in both neutral and alkaline medium. Deuterated water was chosen as a solvent for TRPD experiments due to the convenience of prolonging the lifetime of $O_2(^1\Delta_g)$, as compared with its lifetime in $H_2O$, given the relatively long time-response (ca. 3 μs for the IR detector employed).

As mentioned elsewhere [17], no relevant information about the efficiency of the actual photodegradation reaction is obtained from the reactive rate constant $k_r$ in a $O_2(^1\Delta_g)$-mediated photooxidation. This information is only provided

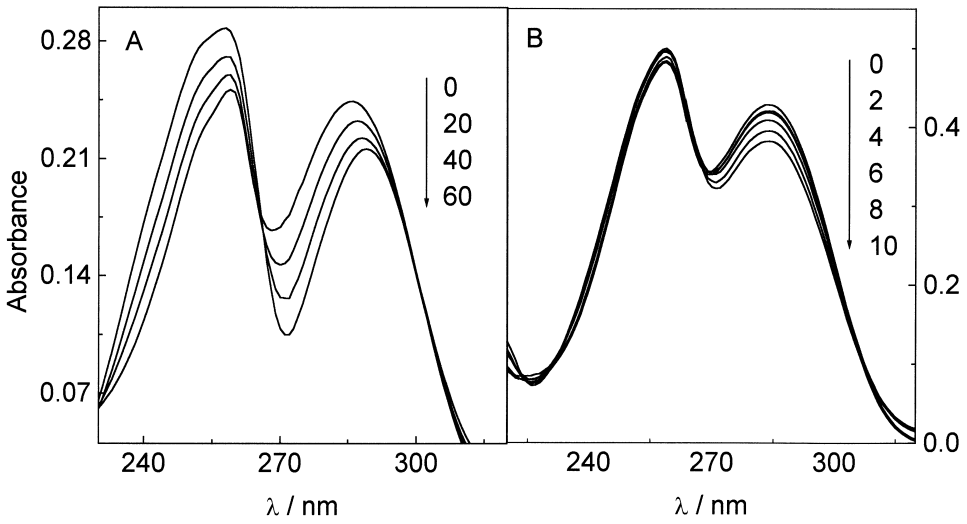



Fig. 3. Spectral changes in the riboflavin-sensitised aerobic (panel A) and anaerobic (panel B) photo-irradiation of sulfathiazole. Absorbance of the sensitiser at 445 nm 0.6. Numbers on the spectra represent irradiation time in minutes.

by the photo-oxidation quantum efficiencies ($\varphi_r$) (Eq. (3)).

$$\varphi_r = k_r[\mathbf{Q}]/(k_d + k_t[\mathbf{Q}]) \quad (3)$$

Expression (3) takes into account the simultaneous effects of both physical and chemical interactions, these being the first contribution usually interpreted in practical terms as a form of self-protection against $O_2(^1\Delta_g)$-mediated photo-oxidation. The describe [18] $k_d$ values in $H_2O$ ($2.5 \times 10^5$ $s^{-1}$) were used for $\varphi_r$ calculations, for a concentration of **Q** of 1 mM. The $\varphi$ values range between 0.002 and 0.31 depending on the particular thiazole and pH of the medium, as are shown in Table 1.

### 3.4. Photoirradiation of Rf and Rf–thiazoles mixtures

Due to solubility reasons, the experiments employing Rf and **Q** were performed in EtOH–$H_2O$ 1:1.

Under conditions similar to those described for RB but using Rf as a sensitiser, the visible-light photoirradiation of mixtures Rf + **Q** resulted in the spectral changes shown in Fig. 3, in the presence of air (panel A) and in nitrogen-bubbled solutions (panel B). Oxygen consumption was detected upon irradiation of such aerated mixtures. The rate of oxygen uptake was either nil or greatly reduced in the comparative irradiations of: (a) a solution of Rf, but in the absence of **Q**; (b) a solution of Rf, but with (**Q**) > 5 mM; and (c) the mixture Rf + 0.01–0.1 mM **Q** and 1 mM sodium azide.

It is known that the photodegradation of Rf in water under visible light irradiation predominantly proceeds through the Rf triplet state [19] and the rate of the process can be estimated by absorption spectroscopy. Competitive irradiations of nitrogen-saturated solutions of Rf in the absence and in the presence of 0.1 mM **Q** showed that this rate was drastically diminished in the presence of **Q** (Fig. 4), and the same effect was observed in air-equilibrated solutions, although much longer irradiation times were necessary in order to obtain measurable absorption changes. These facts clearly indicate the interaction of **Q** with electronically excited states of Rf.

No association between Rf or RB ground states and **Q** was detected using spectroscopy. The difference absorption spectrum of 0.05 mM Rf or RB in the 380–600 nm range was not affected by the presence of **Q** up to 1 mM (results not shown).

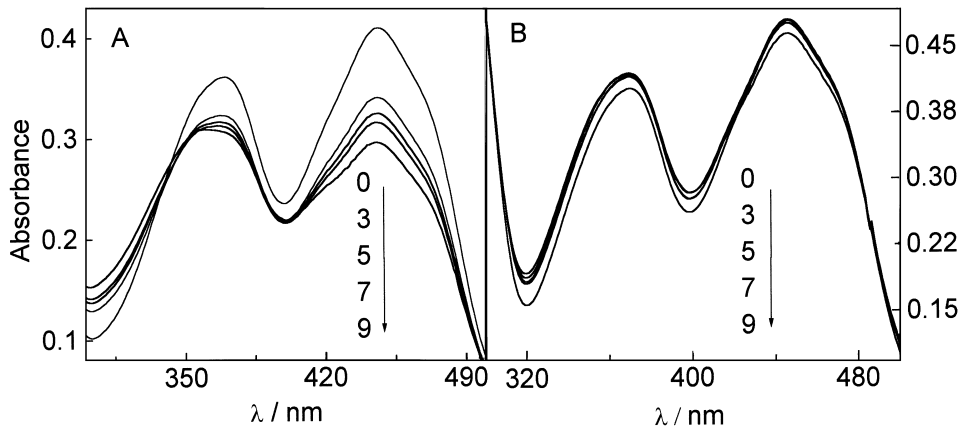



Fig. 4. Absorption spectra of riboflavin in nitrogen-saturated ethanol:water 1:1 (v/v) after different irradiation doses (panel A). In panel B: the same as in panel A, but in the presence of sulfathiazole 0.4 mM. Irradiation wavelength >400 nm. Numbers on the spectra represent irradiation time, in minutes.

### 3.5. Quenching of excited singlet Rf by the thiazoles

Rf exhibits an intense fluorescence emission in air-equilibrated water solution, centered at 515 nm, with a reported [20] quantum yield of 0.25. In the presence of [**Q**] > 4 mM, the stationary fluorescence quenching of Rf is detectable as a decrease in the emission intensity, but the shape of the emission spectrum does not change. Monitoring the fluorescence intensity of Rf in the absence ($I_o$) and in the presence ($I$) of different **Q** concentrations, the classical Stern–Volmer treatment $\left(I_o/I = 1 + {}^1K_{sv}[\mathbf{Q}]\right)$ allows the determination of the Stern–Volmer constant, ${}^1K_{SV}$, from the slope of the plot $I_o/I$ vs. [**Q**] Fig. 5. In the case that the only process responsible Rf fluorescence inhibition were the quenching of Rf singlet excited state, the ${}^1K_{sv}$ value so obtained (11.4 and 8.6 $M^{-1}$ for **STZ** and **SCSTZ** respectively) would be equal to the product $k_{q(1)} \times {}^1\tau_o$ where ${}^1\tau_0$ is the Rf fluorescence lifetime (Scheme 1). The time resolved fluorescence measurements on Rf ${}^1\tau_o$ yielded 5.2 ns, in accordance with published data [21] and, consequently, a $k_{q(1)}$ value of 2.2 and $1.6 \times 10^9$ $M^{-1}$ $s^{-1}$ for **STZ** and **SCSTZ**, respectively, which are close to the diffusion rate constants in aqueous solution [22]. These values are practically the same as those obtained by direct application of the Stern–Volmer equation $\left({}^1\tau_o/{}^1\tau = 1 + k_{q(1)}\,{}^1\tau_o[\mathbf{Q}]\right.$, Fig. 5$\left.\right)$, where ${}^1\tau$ is the Rf fluorescence lifetime in the presence of **Q**.

### 3.6. Quenching of triplet Rf by *Q*

The disappearance of Rf triplet state, generated by a 532 nm laser pulse, was followed from the first-order decay of the absorbance at 680 nm, a zone where the interference from other possible species is negligible. In this way, it was observed that the presence of **Q**, in concentrations up to 0.015 mM, considerably decreases the lifetime of the Rf triplet state. A Stern–Volmer treatment of the triplet quenching, using the expression $1/\tau_3 = 1/\tau_{o3} + k_{q(3)}[\mathbf{Q}]$ (where $\tau_3$ and $\tau_{o3}$ are the experimentally determined triplet lifetimes of Rf in the absence and in the presence of different **Q** concentrations, respectively [Fig. 5(B)], yielded a bimolecular rate constant value for the quenching of triplet Rf by **Q** ($k_{q(3)}$, Scheme 1) of $17 \times 10^8$ $M^{-1}s^{-1}$ and $6.9 \times 10^8$ $M^{-1}$ $s^{-1}$ for **STZ** and **SCSTZ**, respectively.

### 3.7. Microbiological analysis

The microbiological activity of **STZ** visible-light irradiated solutions, in the presence of RB in sensitizing concentrations, was evaluated at pH 6 and 12 by measurement of the diameter of the inhibitory halo before ($S_0$) and after ($S$) photolysis.

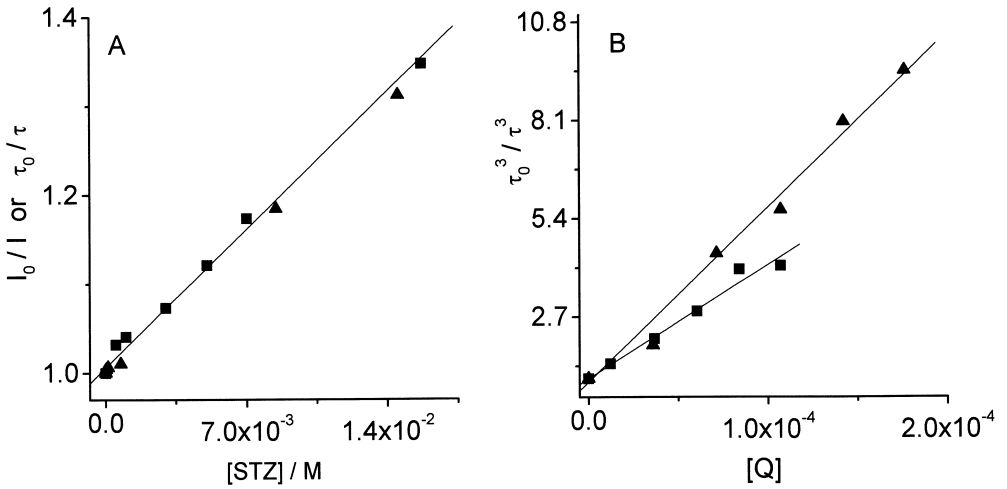



Fig. 5. Stern–Volmer plot for the quenching of riboflavin fluorescence by succinylsulfathiazole (panel A) in air-equilibrated aqueous solution. ${}^1\tau_o/{}^1\tau$ and $I_o/I$ correspond to time-resolved (■) and static (▲) experiments, respectively. Panel B: Stern–Volmer plot for the quenching of riboflavin triplet state by sulfathiazole (▲) and succinylsulfathiazole (■) in nitrogen-saturated aqueous solution. ${}^3\tau$ is the triplet lifetime of riboflavin.

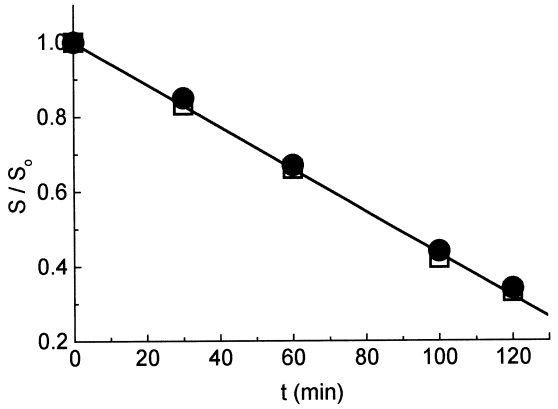



Fig. 6. Relative rates of decrease of the bacteriostatic activity of sulfathiazole at pH 6 (○) and pH 12 (●) upon rose bengal-sensitised photoirradiation. $S$ and $S_0$ represent the diameter of the inhibitory halo in a culture medium of *Bacillus subtilis*.

Results, expressed as relative rates of decrease of the bacteriostatic activity, are shown in Fig. 6.

## 4. Discussion

The evidence above clearly indicates that **STZ** and **SCSTZ** are decomposed through dye-sensitised photoinduced degradation, under different experimental conditions. The mechanism governing this decomposition does not seem to be simple. Nevertheless, as a first approach, two main reaction pathways can be proposed, namely an aerobic process and a group of other interactions that could operate in the absence of dissolved oxygen.

The aerobic process is represented by the decomposition of the thiazoles through a $O_2({}^1\Delta_g)$-mediated process. The two sensitisers used here, RB and Rf, are efficient $O_2({}^1\Delta_g)$ generators, with reported quantum yields in water of 0.7 and 0.49 respectivel [23]. The respective quantum efficiencies of photooxidation of **STZ** and **SCSTZ** were evaluated (Table 1) from the experiments using RB, possibly the dye-sensitiser most frequently employed in kinetic studies of $O_2({}^1\Delta_g)$-mediated processes. The pH of the medium had practically no kinetic effect on the photo-oxidation of **STZ** (see $\varphi_r$ values in Table 1). Alternatively, the presence of the succinyl moiety, in its non-ionized form, highly favours the physical quenching process, whereas the ionization of the carboxylic group promotes effective photo-oxidation. These facts clearly demonstrate the involvement of the aliphatic chain in the reactive pathway.

According to our knowledge, the only report in the literature in relation to thiazole derivatives belongs to Chen and Ho [24], who qualitatively reported reactivity of 2,4,5-trimethyl-thiazole

towards $O_2(^1\Delta_g)$. These authors attributed the reaction to a 1-4-cycloaddition reaction, as previously postulated by Matsura and Saito [25] for five-membered ring compounds. The results obtained here suggest that in the case of the totally unreactive behaviour exhibited by unsubstituted thiazole, the presence of electron donating substituents, represented by three methyl groups, favours the oxidative pathway, as in the case described by Chen and Ho [24]. In this context, the relatively high reactivity of **STZ** and **SCSTZ** towards $O_2(^1\Delta_g)$ could be attributed to the aniline and *N*-substituted aniline moieties, respectively. These groups are effective $O_2(^1\Delta_g)$ quenchers [3], with reported $k_t$ values in the order of $10^8$ $M^{-1}$ $s^{-1}$, very similar to those of the above mentioned (Table 1) for sulfathiazole bacteriostatics.

In the case of Rf as a sensitiser, besides the $O_2(^1\Delta_g)$- mediated mechanism, the second group of competitive reactions operate. These reactions, not necessarily aerobic, include both **Q** and Rf photoconsumption. In the following paragraphs we will discuss, in kinetic terms, the implication of all these processes on the prediction of the chemical fate of the sulfathiazoles and of the isoalloxazine pigment Rf, under different experimental conditions.

The excellent concordance between time-resolved and static experiments on the quenching of the fluorescence of Rf by **Q** (Fig. 5) suggests that the only process involved is an interaction between excited singlet Rf and **Q**. Furthermore, the linearity of the plots corroborates the absence of associations between the corresponding ground states, already indicated by UV absorption measurements. Moreover, the kinetic data indicate that the singlet quenching process must not affect to a considerable extent the population of Rf triplet state (Scheme 1), because the excited singlet Rf, with a lifetime of 5.2 ns, can be hardly quenched by **Q** in the concentrations herein employed (ca. 0.1 mM). For [**Q**] > 1 mM, the massive quenching of excited singlet Rf prevents the formation of triplet Rf and, hence, of $O_2(^1\Delta_g)$, as the oxygen uptake experiments indicate.

The triplet excited state of Rf is quenched by **STZ** and **SCSTZ** with rate constants $k_{q(3)}$ of $17 \times 10^8$ and $6.9 \times 10^8$ $M^{-1}$ $s^{-1}$ (Scheme 1, process 3), as determined by flash photolysis. This indicates that in the absence of oxygen and in the presence of 0.1 mM **Q**, Rf, photodecomposition via triplet state is prevented (Fig. 4). As a consequence, in air saturated solutions, the deactivation process could compete with the generation of $O_2(^1\Delta_g)$.

It is known [8,26,27] that Rf decomposes via its triplet state, and via $O_2(^1\Delta_g)$ reaction. Nevertheless, with typical Rf sensitizing concentrations (ca. 0.005 mM), and considering [7] a $k_{rRf}$ value of $6 \times 10^7$ $M^{-1}$ $s^{-1}$, the singlet oxygen-mediated degradation of Rf should be greatly inhibited in the presence of **Q** in concentrations of the order of 0.1 mM or higher. In other words, in most of the cases $(k_q + k_r)[\mathbf{Q}] \geqslant k_{rRf}[Rf]$.

Finally, the experimental results indicate a clear decrease of the bacteriostatic activity in the visible-light photoirradiated **STZ**–RB solutions. The coincidence between the relative microbiological rates of photolized **STZ** solutions in slightly acidic and alkaline pH is in agreement with the very similar $\varphi_r$ exhibited by the thiazole derivative in both media. Furthermore, the observed behaviour suggests that the photoproducts of **STZ** degradation do not posses any activity against *Bacillus subtilis*, which was employed in our tests.

## 5. Conclusions

It is evident that the RB and Rf sensitise the photodegradation of **STZ** and **SCSTZ** in air-equilibrated aqueous solutions. In the case of RB the dominating mechanism is the $O_2(^1\Delta_g)$-mediated photo-oxidation. Employing Rf as a dye sensitiser, visible light irradiation triggers a complex mixture of physical and chemical processes, the relative predominance of which depends on the relative concentrations of Rf and the bacteriostatics. With ca. 0.01 mM Rf and thiazoles $\leqslant$0.01 mM, the dominant pathway is the generation of $O_2(^1\Delta_g)$ and the concomitant photo-oxidation of the bacteriostatics. Under similar conditions, but with ca. 0.1 mM **Q**, competitive reactions of triplet Rf with both $O_2\left(^3\sum_g^-\right)$ and **Q** are operative, giving rise to the inhibition of the Rf photodegradation. In the

presence of much higher **Q** concentrations ($\geqslant 1$ mM), the quenching of excited singlet Rf is the dominant process, and the photodegradation of Rf is thus prevented. The $O_2(^1\Delta_g)$-mediated photo-oxidation produces a clear decrease in the microbiological activity of **STZ**.

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